FOODBORNE PATHOGENS, MASTITIS, MILK QUALITY, AND DAIRY FOOD SAFETY

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Introduction

We hear much these days about food safety. What's the concern? More than 200 known diseases are transmitted through food by a variety of agents that include bacteria, fungi, viruses, and parasites. According to public health and food safety experts, each year millions of illnesses in this country and throughout the world can be traced to foodborne pathogens. While the food supply in the United States is one of the safest in the world, the Center for Disease Control and Prevention (CDC, 2003; CDC, 2004) estimates that 76 million people get sick, more than 300,000 are hospitalized, and 5,000 Americans die each year from foodborne illness. The risk of foodborne illness has increased markedly over the last 20 years, with nearly a quarter of the population at higher risk for illness today. Consequently, preventing illness and death associated with foodborne pathogens remains a major public health challenge.

Why has the risk of foodborne illness increased? There are several reasons. Much has changed in what we eat and where we eat. A greater variety of foods are consumed, particularly seafood, fresh fruit, and fresh vegetables, and consumers demand these foods year round. To satisfy this demand, more foods are imported from foreign countries. Another factor is that more meals are eaten away from home. As more people become involved in preparing our meals, the chance for foodborne illness increases dramatically. In addition, the threats are numerous and varied such as *Escherichia coli* O157:H7 in meat and apple juice; *Salmonella* in meat, eggs, on vegetables and poultry; *Vibrio* in shellfish; *Cyclospora* on fruit; and *Cryptosporidium* in drinking water. These pathogens can be deadly, especially for people at highest risk. This situation becomes even more problematic because of rapidly changing demographics, with an increasing number of elderly people and immunocompromised individuals who are more susceptible to foodborne pathogens (Notermans and Hoogenboom-Verdegaal, 1992).

The economic impact of foodborne diseases on society is staggering. In 1993, the Economic Research Service (ERS) of USDA indicated that the annual cost of human disease caused by the more common foodborne pathogens ranged from $5.6 to $9.4 billion dollars (Busby and Roberts, 1995). The number of cases of foodborne disease caused by *E. coli* O157:H7 ranged between 8,000 to 16,000 with 400 deaths and a cost between $200 to $600 million dollars. For *Salmonella* species, the number of cases ranged form 696,000 to 3,840,000 with 3,840 deaths and an estimated cost between $600 million to $3.5 billions dollars (Busby and Roberts, 1995). According to the latest USDA ERS estimates, medical costs, productivity losses and value of premature deaths for diseases caused by five major foodborne bacterial pathogens approach $7 billion per year. Cost estimates for 2000 were $1.2 billion for *Campylobacter* (all serotypes), $2.4 billion for *Salmonella* (nontyphoidal), $0.7 billion for *E. coli* O157:H7, $0.3 billion for non-O157 Shiga-toxin producing *E. coli*, and $2.3 billion for *Listeria monocytogenes*. It is therefore evident that reducing foodborne pathogen contamination of our food supply could save both lives and billions of dollars in costs annually.

Should the dairy industry be concerned about food safety? You bet, and there are several good reasons why such as: (1) bulk tank milk contains several foodborne pathogens that cause human disease, (2) outbreaks of disease in humans have been traced to the consumption of raw unpasteurized milk and have also been traced back to pasteurized milk, (3) raw unpasteurized milk is consumed directly by dairy producers and their families, farm employees and their families, neighbors, etc., (4) raw unpasteurized milk is consumed directly by a much larger segment of the population via consumption of several types of cheeses including ethnic cheeses manufactured from unpasteurized raw milk, (5) entry of foodborne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms and subsequent contamination of processed food products, (6) pasteurization may not destroy ALL foodborne pathogens in milk, and (7) faulty pasteurization will not destroy all foodborne pathogens.

The purpose of this communication is to review literature published on the prevalence of foodborne pathogens (primarily *Campylobacter jejuni,* Shiga-toxin producing *E. coli* (STEC)*, L. monocytogenes*, and *Salmonella*) in milk and in the dairy environment, the role of foodborne pathogens as mastitis-causing bacteria and their impact on milk quality, and to discuss public health and food safety issues associated with foodborne pathogens found in the dairy environment.

<u>Prevalence of Foodborne Pathogens in Milk</u>

Several surveys have detected foodborne pathogens in bulk tank milk (Davidson, 1989; Doyle and Roman, 1982; Farber et al., 1988; Fedio and Jackson, 1990; Hassan et al., 2000; Jayarao and Henning , 2001; Liewen and Plautz, 1988; Lovett et al., 1983; Lovett et al., 1987; McEwen et al., 1988; McManus and Lanier, 1987; Murinda et al., 2002a; 2002b; 2004a; 2004b; Rohrbach et al., 1992; Slade et al., 1988; Steele et al., 1997; Van Kessel et al., 2004; and Waak et al., 2002). Results of those studies have shown clearly that prevalence rates of foodborne pathogens including *C. jejuni*, STEC*, L. monocytogenes,* and *Salmonella* spp. in milk vary considerably (Tables 1 and 2).

Numerous factors likely contribute to the variation observed such as geographical location, season, farm size, number of animals on the farm, hygiene, farm management practices, variation in sampling, variation in types of samples evaluated, and differences in detection methodologies used. However, in spite of the variation, all of these surveys demonstrated quite clearly that milk can be a significant source of foodborne pathogens of human health significance.

Rohrbach et al. (1992) reported that the frequency of isolation of foodborne pathogens from 292 bulk tank milk samples from dairies in east Tennessee and southwest Virginia was 12.3% *for C. jejuni,* 8.9% for *Salmonella* species, 4.1% for *L. monocytogenes,* and 15.1% for *Yersinia enterocolitica.* One or more foodborne pathogens were isolated from 32.5% of bulk tank milk samples evaluated. One of the four foodborne pathogens was isolated from 73 of 95 positive

Table 1. Surveys on the isolation of *Campylobacter jejuni* and Shiga-toxin producing *Escherichia coli* from bulk tank milk.

Campylobacter jejuni	0.9	Doyle & Roman (1982)
	1.5	Lovett et al. (1983)
	0.4	McManus & Lanier (1987)
	1.2	Davidson et al. (1989)
	12.3	Rohrbach et al. (1992)
	0.5	Steele et al. (1997)
	9.2	Jayarao & Henning (2001)
Shiga-toxin producing *Escherichia coli*	0.9	Steele et al. (1997)
	3.8	Jayarao & Henning (2001)
	0.8	Murinda et al. (2002b)

Table 2. Surveys on the isolation of *Listeria monocytogenes* and *Salmonella* spp. from bulk tank milk.

Foodborne pathogen	Isolation rate (%)	Reference
Listeria monocytogenes	4.2	Lovett et al. (1987)
	1.3	Farber et al. (1988)
	5.4	Slade et al. (1988)
	4.0	Liewen & Plautz (1988)
	1.6	Davidson et al. (1989)
	1.9	Fedio & Jackson (1990)
	4.1	Rohrbach et al. (1992)
	2.7	Steele et al. (1997)
	4.6	Jayarao and Henning (2001)
	12.6	Hassan et al. (2000)
	1.0	Waak et al. (2002)
	4.9 to 7.0	Muraoka et al. (2003)
	6.5	Van Kessel et al. (2004)
Salmonella spp.	4.7	McManus & Lanier (1987)
	2.9	McEwen et al. (1988)
	8.9	Rohrbach et al. (1992)
	0.2	Steele et al. (1997)
	6.1	Jayarao and Henning (2001)
	1.5	Hassan et al. (2000)
	2.2	Murinda et al. (2002a)
	2.6	Van Kessel et al. (2004)

samples, and 22 samples contained two or more foodborne pathogens. Grade classification of the dairy, milking facilities, barn type, milking hygiene, reported incidence of clinical mastitis among cows, or the number of cows per farm were not significantly associated with the isolation of foodborne pathogens in bulk tank milk. Of 84 dairy producers who used teat disinfection and antibiotic dry cow therapy that were classified as having good milking hygiene, 29 (35%) had bulk tank milk contaminated with foodborne pathogen(s) compared to 12 of 29 (31%) dairies with poor milking hygiene (odds ratio 1.2, *P* = 0.7). Almost 35% of dairy producers who participated, indicated that they consumed raw milk produced on their farm, and 25% of the bulk tank milks from these farms were contaminated with one or more foodborne pathogens (Rohrbach et al., 1992).

In a similar study, bulk tank milk from 131 dairy herds in eastern South Dakota and western Minnesota was examined for the presence of foodborne pathogens (Jayarao and Henning, 2001). Thirty-five of 131 (26.7%) bulk tank milk samples contained one or more species of pathogenic bacteria. *Campylobacter jejuni*, STEC, *L. monocytogenes, Salmonella* spp., and *Y. enterocolitica* were detected in 9.2, 3.8, 4.6, 6.1, and 6.1% of bulk tank milk samples, respectively. Isolates of *Salmonella* belonged to group D (n = 4), B (n = 2), C (n = 1), and E (n = 1) "O" serogroups. All six isolates of *Listeria monocytogenes* were identified as O antigen type 1. Four of five isolates of *E. coli* encoded for the Shiga-toxin 2 gene, while one strain encoded for the Shiga-toxin 1 gene. *Escherichia coli* O157:H7 was not isolated from bulk tank milk samples. Manufacturing grade raw milk producers were at a higher risk (odds ratio 4.98; confidence interval, 1.96 to 12.22) of having one or more foodborne pathogens in their bulk tank milk than were Grade A milk producers. Twenty-one of 79 (26.6%) dairy producers who consumed raw milk produced on their farm had one or more pathogenic bacteria in their bulk tank milk.

More recently, Van Kessel et al. (2004) reported results on the prevalence of foodborne pathogens in bulk tank milk samples obtained as part of the National Animal Health Monitoring System Dairy 2002 survey. The objective of this study was to determine the national prevalence of *Salmonella*, *L. monocytogenes*, and fecal coliforms in bulk tank milk in the United States. Bulk tank milk samples (n=861) were collected from farms in 21 states. Coliforms were detected in 95% (818 of 860) of samples, and the average somatic cell count (SCC) was 295,000 cells/ml. Twenty-two samples (2.6%) were culture-positive for *Salmonella*, and 9 serotypes were identified: Montevideo (n = 7), Newport (n = 4), Muenster (n = 2), Meleagridis (n = 2), Cerro (n = 2), 44:Z36 (Z38) (n = 2), Dublin (n = 1), Anatum (n = 1), and 9, 12:nonmotile (n = 1). *Listeria monocytogenes* was isolated from 56 (6.5%) samples, and serotyping of these isolates yielded 5 serotypes (1/2a, 1/2b, 3b, 4b, and 4c). Of the *L. monocytogenes* isolates, 93% were serotypes 1/2a, 1/2b, and 4b, the most common human clinical isolates. There were no apparent relationships between SCC or specific plate count and incidence of *Salmonella* or *L. monocytogenes*.

Another pathogen that is found frequently in bulk tank milk and is a significant cause of mastitis in dairy cows throughout the world is *Staphylococcus aureus.* Tondo et al. (2000) conducted a study on a milk processing plant to determine the source of *Staph. aureus* contamination and showed that contaminated raw milk was the main source of contamination of the final dairy products. The bovine mammary gland can be a significant reservoir of enterotoxigenic strains of *Staph. aureus.* Enterotoxins produced by enterotoxigenic strains of *Staph. aureus* are classified according to serotypes into A-H and toxic shock syndrome toxin (TSST)-l. The frequency of enterotoxigenicity amongst staphylococcal strains is highly variable (Genigeorgis, 1989; Mossel and Van Netten, 1990). Studies on *Staph. aureus* isolated from cows showed enterotoxigenicity ranging from 0 to 56.5% (Bennet et al., 1986; Castro et al. 1986; Kenny et al., 1993; Masud et al., 1993; Ruzickova, 1994). *Staphylococcus aureus* was isolated from 183 of 300 raw milk samples at a milk cooperative in Kenya and 72 of 97 (74.2%) isolates produced one or more enterotoxins. It was inferred that raw milk was a potential source of enterotoxigenic *Staph.*

aureus in milk and milk products (Ombui et al., 1992). Adesiyun et al. (1998) studied the prevalence of *Staph. aureus* in bulk milk collected from dairy farms in Trinidad. Mean counts of *Staph. aureus* in bulk milk ranged from 5,900 to 12,000 cfu/ml. A total of 105 strains of *Staph. aureus* from bulk milk were tested for production of enterotoxins, 45 of 105 (42.9%) produced Staphylococcal enterotoxin A, B, C, D or a combination of these toxins. The findings of this study concluded that bulk tank milk containing relatively high counts of enterotoxigenic *Staph. aureus* may constitute a health hazard to consumers.

Takeuchi et al. (1998) examined *Staph. aureus* isolated from cows with mastitis and farm bulk milk samples. They reported that TSST was detected in 25 of 43 (58.1%) *Staph. aureus* isolated in milk from cows with clinical mastitis, 79 of 103 (76.7%) from cows with subclinical mastitis, and 85 of 126 (67.5 %) *Staph. aureus* isolated from farm bulk tank milk. The TSST from bovine isolates had similar molecular weight and isoelectric point to that from human isolates. Lee et al. (1998) applied a PCR-based assay to detect toxin genes of staphylococcal isolates from cases of bovine mastitis and observed that none of these isolates encoded for enterotoxins or TSST-1. Conversely, 4 of 13 isolates from Washington State and 6 of 20 isolates from Korea expressed enterotoxins. The study by Lee et al. (1998) demonstrated genetic variation of enterotoxin genes in that there are significant differences in toxicity of mastitis isolates with reference to geographical locations. Enterotoxigenic strains of *Staph. aureus* have been reported to cause a number of diseases or food poisoning outbreaks because of ingestion of contaminated dairy products or milk (Adesiyun et al., 1998; Asao et al., 2003; Genigeorgis, 1989). The most recent large-scale outbreak occurred during June 2000 in Japan and it was caused by consumption of low-fat milk produced from skim milk powder contaminated with *Staph. aureus* enterotoxin A (Asao et al., 2003).

The prevalence of *C. jejuni* in bulk tank milk has been reported to range from < 1% to > 13% (Table 1). *Campylobacter jejuni* is the most frequently identified cause of acute infectious diarrhea in developed countries and is the most commonly isolated bacterial intestinal human pathogen in the United States. Foodborne illness caused by *Campylobacter* is characterized by sporadic cases of chronic gastritis, enterocolitis, and septicemia. About 2.4 to 4 million cases of campylobacteriosis associated with 120 deaths occur each year (Mead et al. 1999). Foodborne infections by *Campylobacter* can result in *Campylobacter*-associated Guillian-Barré Syndrome. Humans get infected through ingestion of untreated water, contaminated non-pasteurized milk, and milk not properly pasteurized (Evans et al., 1996; Fashey et al., 1995). *Campylobacter jejuni/coli* are excreted through feces and animal secretions and dairy cattle get infected through ingestion of water and feeds contaminated with manure. *Campylobacter jejuni* can cause mastitis in cows and it can be shed in milk of carrier asymptomatic cows. Direct milk excretion of *C. jejuni/coli* by clinically healthy cows has been described and implicated in the etiology of human enteritis following consumption of contaminated milk (Orr et al., 1995). Large outbreaks due to *Campylobacter* have been associated with drinking unpasteurized milk or contaminated water. Cow manure is a principal reservoir and farm practices using manure as fertilizer on cropland are considered a risk factor for occurrence of *Campylobacter* foodborne disease.

Far less is known about the prevalence of STEC in milk than the other major foodborne pathogens. STEC are of immense economic and public health significance. STEC O157:H7 are characterized by low infectious doses, 1-100 colony-forming units (Paton and Paton, 1998). STEC are highly pathogenic in humans where they cause serious acute illness and long-term sequelae (Karmali, 1989; Nataro and Kaper, 1998; Paton and Paton, 1998). Manifestations of illnesses caused by STEC that are linked to production of Shiga toxins include, non-bloody diarrhea, diarrhea-associated hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (Karmali, 1989). HUS cases in North America are predominantly caused by O157:H7 STEC. Enterohemorrhagic *E. coli* (EHEC) strains constitute a subtype of STEC serotypes that have been firmly associated with bloody diarrhea and HUS. EHEC are generally more pathogenic than other STEC because they possess a fuller complement of virulence determinants that encode production of intimin and enterohemolysin (Karmali, 2004). Serotypes O26, O111, O103, and especially O157, have been the predominantly isolated EHEC (Nataro and Kaper, 1998).

Of the few reports published, the prevalence of STEC in bulk tank milk has been reported to occur in 0.8% to 3.8% of samples evaluated (Table 1). Murinda et al. (2002b) reported the detection of *E. coli* O157:H7 from 8 of 30 (26.7%) dairy farms at different sampling times. Two of 268 (0.75%) bulk tank milk samples and 8 of 415 (1.93%) cull dairy cow fecal samples tested positive for *E. coli* O157:H7. Murinda et al. (2004b) isolated O26, O111, O103, and O157:H7 EHEC serotypes from dairy cows and/or the dairy farm environment. It appears that these serotypes could be of epidemiological significance in the United States. However, since there are presently no well-defined serotype-specific methods for their routine isolation, the epidemiological significance of non-O157:H7 STEC remains undefined.

The prevalence of *L. monocytogenes* in bulk tank milk has been reported to range from 1 to > 12% (Table 2). *Listeria monocytogenes* causes septicemia and meningitis in humans. Pregnant women are particularly susceptible since *L. monocytogenes* infection may result in spontaneous abortions or stillbirth of the fetus. *Listeria monocytogenes* has been isolated from mammals, birds, fish, crustaceans and insects. In addition, *Listeria* species are widespread in nature and live naturally in plants and soil environments. It can grow in a wide range of temperature and pH. This adaptability enables *Listeria* to grow in refrigerated raw milk and in low quality silage with a pH > 4.5. At high bacterial concentrations, *L. monocytogenes* can survive minimum HTST pasteurization (Bunning et al., 1988). *Listeria monocytogenes* can cause mastitis in cows and it can be shed in milk of carrier asymptomatic cows. Human contamination occurs through consumption of raw milk or products manufactured with raw milk or through ingestion of processed food cross-contaminated with pathogens present in the food processing plant environment (Gravani, 1999). In cattle, *L. monocytogenes* can cause neurological disease, abortion, or no symptoms of disease. Healthy, but infected animals, shed *Listeria* in feces and fecal contamination of pastures or vegetables was incriminated as a source of contamination for humans and ruminants. Therefore, spreading of untreated manure onto pastures and cropland was regarded as a risk factor for *Listeria* foodborne disease.

The prevalence of *Salmonella* species in bulk tank milk has been reported to range from < 1% to almost 9% (Table 2). *Salmonella* species have been linked with illness in animals and humans, and are one of the most commonly reported causes of human foodborne disease (Bean and Griffin, 1990). *Salmonella* live in the intestinal track of various animal species, including cattle, and therefore represent a major reservoir for human foodborne disease. Humans get infected primarily via fecal contamination of food products or water, however, direct contact with ill animals is also another source of contamination, especially for farm families. A high percentage of human salmonellosis occurs through consumption of raw milk or dairy products manufactured with raw milk.

Several *Salmonella* serotypes have been isolated from clinically ill cattle. *Salmonella enterica* Serotype Typhimurium definitive type 104 (DT 104) is of particular concern to animal and public health agencies because of its multiple antibiotic resistance (Besser et al., 1996). In-line milk filters from farms located in central, east, north, and west regions of New York State were evaluated to determine the prevalence of *Salmonella* in New York dairy herds. Six of 404 (1.5%) milk filters were positive for *Salmonella* spp. From these isolates, one was confirmed as *Salmonella enterica* Serotype Typhimurium DT 104 (Hassan et al., 2000). In another study conducted on 12 dairy farms from Minnesota, Michigan, New York and Wisconsin where fecal, bulk tank milk and environmental samples were analyzed for *Salmonella*, it was found that 1.1% of bulk tank milk (n = 91) and 12.6% of in-line milk filter samples (n = 87) were positive for *Salmonella* spp. The higher percentage of isolation from environmental samples corresponded to sick cow pen floor (26.3 %, n = 57), calving pen floor (16.7 %, n = 72), milking cow feed bunk (16.3%, n = 92), lagoon or other manure storage (15.6%, n = 90), and cow water tank or drinking cups (13.5 %, n = 89). From 4049 fecal samples tested, 9.3% were *Salmonella*-positive and the percentage of positive samples per farm ranged from 0.3 to 28%. From 811 environmental samples, 12.9 % were positive for *Salmonella* spp. with a range from 0 to 40 % per farm (Warnick et al., 2003).

Prevalence of Foodborne Pathogens in the Dairy Environment

The origin of foodborne pathogens can be from excretion from the udder of an infected animal, or through direct contact with infected sources in the environment. Most foodborne pathogens inhabit the ruminant intestinal tract, and therefore, dairy cattle are considered a major reservoir of *Salmonella*, *Campylobacter,* and STEC. *Listeria* species are widespread in nature and live naturally in plants and soil environments. Epidemiological studies have shown that cattle probably get infected through consumption of water and feedstuffs contaminated with feces and other cattle secretions/excretions. Presence of foodborne pathogens in bulk tank milk seems to be directly linked to fecal contamination that occurs primarily during the harvesting of raw milk, however, some foodborne pathogens can cause mastitis in which the organism was directly excreted into milk. Introduction of raw milk contaminated with foodborne pathogens into processing plants and persistence in biofilms represents an important risk of post-pasteurization contamination that could lead to exposure of the consumer to pathogenic bacteria. (Arizcun et al., 1998; Roberts and Wiedmann, 2003; Wong, 1998).

Dairy farms are an important reservoir of foodborne pathogens. The microaerophilic and thermophilic nature of *Campylobacter jejuni/coli* hampers growth of this organism in feed or in nature. In spite of growth restrictions, *C. jejuni/coli* are very versatile and metabolically active organisms capable of exploiting a variety of environments, especially the intestinal tract of warm-blooded mammals and birds. Enumeration studies showed that a critical amplification stage in the *Campylobacter* cell cycle occurs in the intestines of carrier animals. Once cells are excreted to the environment, they must evolve survival strategies until ingested by a susceptible host. Thus, the intestinal tract and feces of susceptible (carrier) animals are considered the major reservoir of this foodborne pathogen. A similar theme was described for *L. monocytogenes*. Data reported by Nightingale et al. (2004) support the model in which the presence of the pathogen depends on the ingestion of contaminated feed followed by amplification in bovine hosts and fecal dissemination in the farm environment. A similar series of events seems to occur with STEC. The influence of the diet (grains vs. forage) on the shedding of STEC in feces suggests that an amplification stage also occurs in the GI tract of ruminants. The terminal rectum region of the GI tract was an important site where this pathogen showed specific tropism (Naylor et al., 2003). *Escherichia coli* O157:H7 was detected in the terminal rectum region regardless if animals were experimentally or naturally infected. The pathogen was detected in feces up to 4 weeks after experimental inoculation or 22 days in those that cohabited with infected animals (Naylor et al., 2003). The authors described the finding of a naturally infected calf with positive detection of *E. coli* O157: H7 in the terminal rectum mucosa. This calf was detected in a straw court from where 16 of 35 calves were positive for *E. coli* O157:H7 in feces from which 3 calves showed high numbers of this pathogen (> 10^4 cfu/g) in feces. These findings lead authors to propose the existence of "super-shedders" and colonization of the terminal rectum was a condition for this status (Naylor et al., 2003). Taken together, colonization of the GI tract of the bovine and amplification of *C. jejuni/coli, L. monocytogenes* and *E. coli* O157: H7 appears to be a required stage in the cell cycle of these foodborne pathogens. Shedding of foodborne pathogens in feces and distribution in the environment where cows live assures animal re-infection and persistence of the pathogen on the farm. This together with the infection of other warm-blooded mammals, birds and insects that live on the farm place these production units as major reservoirs for foodborne pathogens.

A recent study from our laboratory was conducted to investigate the major habitats of pathogens on dairy farms that could act as reservoirs or transient carriers of the 5 major foodborne pathogen groups, namely, *Salmonella* spp., *L. monocytogenes*, *C. jejuni,* and O157 and non-O157 STEC (Murinda et al., 2004b). Six visits were conducted to 4 dairy farms to collect swab, liquid and solid dairy farm environmental samples (165 to 180/farm; 15 sample types). Pathogens were isolated on agar media, typed biochemically, and confirmed using multiplex polymerase chain reaction protocols. *Campylobacter jejuni*, *Salmonella* spp. and *L. monocytogenes*, sorbitol-negative (SN)-STEC O157:H7 and sorbitol-positive (SP)-STEC, respectively, were isolated from 5.1%, 3.8%, 6.5%, 0.7%, and 17.3% of samples evaluated (Table 3). Whereas other pathogens were isolated from all 4 farms, SN-STEC O157:H7 were isolated from only two farms. Diverse serotypes of SP-STEC including O157:H7, O26:H11, O111, and O103 were isolated. None of the five pathogen groups studied were isolated from bulk tank milk. Most foodborne pathogens (44.2%) were isolated directly from fecal samples. Bovine fecal samples, lagoon water, bedding, bird droppings and rats constituted areas of major concern on dairy farms. Although in-line milk filters from two farms tested positive for *Salmonella* or *L. monocytogenes*, none of the pathogens were detected in the corresponding bulk tank milk samples.

Among the four farms studied, farm A had the best rodent and manure-management practices; and this appeared to be reflected in fewer pathogens isolated (59.4% - 61.5% less) than farm B, C and D (Murinda et al., 2004b). As in our earlier studies (Murinda et al., 2002a; 2002b), Farm D was positive for both STEC O157: H7 and *Salmonella* spp., while farm C was negative for STEC O157:H7 and positive for *Salmonella*. These data appear to indicate persistence of these pathogens on both farms and presence of locally cycling populations. Farm A was positive for both pathogens in the first study, although in the subsequent study, it was O157:H7 STEC-negative, but *Salmonella*-positive. Farm A had the most improved manure management practices and had eradicated rats, which were rampant in the first study. On the other hand, farm B was negative for both pathogens in the first study, but was *Salmonella* and SN-STEC O157:H7-positive in the subsequent study; this might suggest a deterioration of management conditions at this farm. Our observations at farm B support these inferences. The retrogression of farm B from negative to positive was correlated to certain poor management practices. Visually there were infrequent changes of heavily manure-laden straw bedding and frequent changes in milking staff. Furthermore, due to off-farm commitments, the farm owner spent less time on the farm than during the first study. Good manure management practices, including control of feral animals, are critical in assuring dairy farm hygiene.

Table 3: Isolation of foodborne pathogens from the dairy farm environment of four dairies[1].

Sample type	Number of samples	Foodborne pathogens isolated			
		L. monocytogenes	*Salmonella*	*C. jejuni*	*SN-STEC O157:H7*
Feed/silage	97	6	3	1	0
Trough water	92	1	3	0	0
Lagoon water	94	7	2	13	0
Fecal slurry/pats	98	14	4	7	2
Calf fecal swab	86	1	2	4	1
Heifer fecal swab	4	0	0	1	0
Bedding	90	13	6	5	0
Floor/milking Parlor	10	0	0	3	0
Bulk tank milk	49	0	0	0	0
In-line milk filters	24	1	1	0	0
Milking parlor hose water	17	0	0	0	0
Bird droppings (mostly geese)	20	2	2	1	2
Flies	5	0	0	0	0
Rats	4	0	3	0	0
Birds	1	0	0	0	0
Total	691	45 (6.5%)	26 (3.8%)	35 (5.1%)	5 (0.7%)

[1] From Murinda et al., 2004b.

Several reports indicated that cattle are a major reservoir of STEC and feces was the main vehicle for contamination of raw food (milk and beef) produced on dairy farms. STEC O157:H7 serotype has been isolated frequently from cattle feces, and most human EHEC O157:H7 infections originate, either directly or indirectly, from this source (Besser et al., 1997; 2001). Several investigations aimed at the identification of possible intervention strategies to control the prevalence of *E. coli* O157:H7 have linked production practices (critical points) with persistence of this foodborne pathogen in cattle and generation of reservoirs in the farm environment (Elder et al., 2000; Garber et al., 1995; Hancock et al., 1997; Shere et al., 1998; Zhao et al., 1995). Among these, diet (Buchko et al., 2000; Cray et al., 1998; Diez-Gonzales et al., 1998; Herriot et al., 1998), age of cattle (Cray and Moon, 1995; Garber et al., 1995), management of manure and fecal slurry, contaminated animal drinking water (Faith et al., 1996; Shere et al., 1998), and management of pre-and postweaned calves (Faith et al., 1996; Garber et al., 1995; Shere et al., 1998) have been identified as risk factors for infection and shedding of *E. coli* O157:H7 by cattle. Especially important is the use of manure as a fertilizer or contaminated water to irrigate field crops. Contaminated manure and irrigation water were probable vehicles for the pathogen in many human disease outbreaks. Supporting data was obtained from a study where the occurrence and persistence of *E. coli* O157:H7 was determined on lettuce and parsley grown in soil fertilized with contaminated poultry or bovine manure composts or treated with contaminated irrigation water. Result from this study indicated that *E. coli* O157:H7 could persist for 154 to 217 days in soils fertilized with contaminated composts. After seedlings were planted, *E. coli* O157:H7 could be detected on lettuce and parsley for up to 77 and 177 days, respectively. In addition, *E. coli* O157:H7 persisted in soil for more than 5 months after application of contaminated compost or irrigation water, regardless of source or crop type (Islam et al., 2004).

Foodborne Pathogens, Mastitis, and Milk Quality

Staphylococcus aureus continues to be the most prevalent contagious mastitis pathogen worldwide. Mastitis prevention and control practices targeted at *Staph. aureus* have been shown to reduce the incidence on *Staph. aureus* intramammary infections. However, mastitis prevention and control practices need to be followed rigorously and applied continuously to achieve successful control of *Staph. aureus* intramammary infections. To add to the complexity of *Staph. aureus* mastitis, introduction of apparently healthy lactating cows and heifers can introduce *Staph. aureus* into a herd. This could result in a new or exacerbate an existing *Staph. aureus* problem in the herd. The biology of *Staph. aureus* has been fairly well understood both at the genetic and functional level, however, attempts to translate this knowledge to develop an effective vaccine for the prevention of *Staph. aureus* mastitis has met with limited success. Very recent evidence also suggests that *Streptococcus agalactiae*, another common contagious mastitis pathogen which has long been recognized as an important cause of mastitis in dairy cows, may be associated with human neonatal infection (Bisharat et al., 2004). This topic and its implications will be reviewed elsewhere (Leigh, 2005) at the Conference Symposium on Does High Somatic Cell Counts in Milk Constitute a Human Health Risk?

Some foodborne pathogens can cause mastitis in dairy cows and impact milk quality. *Campylobacter jejuni* has been isolated from cows with mastitis. However, this organism is isolated infrequently and does not appear to be a significant cause of bovine mastitis. Lander and Gill (1980) experimentally infected 5 mammary quarters of two lactating cows by intramammary inoculation of *C. coli/jejuni* in doses ranging from 2.6 colony forming units (cfu) to 3.8×10^9 cfu. Infected mammary quarters developed clinical mastitis and *Campylobacters* were isolated in large numbers from milk of experimentally infected mammary glands. Milk from uninfected mammary quarters, and blood and feces remained free of the organisms. *Campylobacter* could only be isolated by incubation of culture plates in a microaerophilic atmosphere. Results of this study demonstrated that *C. coli/jejuni* can cause mastitis in the cow and suggests that the bovine udder is a potential source of *C. coli/jejuni* in raw milk. Gudmundson and Chirino-Trejo (1993) indicated that *C. jejuni* mastitis occurred in a Holstein cow 60 days into the first lactation. The infection was characterized by a sudden onset, pyrexia, a painful mammary quarter and pink milk with a few small clots present. A treatment protocol consisting of parenteral oxytetracycline, frequent stripping and intramammary infusions of erythromycin was effective.

Hutchinson et al. (1985) described a community outbreak of *Campylobacter* enteritis associated with consumption of raw milk, apparently contaminated by two cows with *Campylobacter* mastitis. The outbreak occurred in two phases. Strains of *C. jejuni* of the Penner serogroup complex 4, 13, 16, 50 and Preston biotype code 6100 were isolated from patients in both episodes and from the feces of cattle, milk filters, bulk milk and retail milk. Milk samples from two of 40 milking cows were found to contain *C. jejuni*, and whey samples from these two cows had high *C. jejuni* antibody titres. Waterman et al. (1984) evaluated samples of milk from 1501 cows with mastitis and all were negative for *C. jejuni*. However, feces of 74 apparently healthy Friesian cows were screened for *C. jejuni* and 13% of samples were positive during the summer when the cows were on pasture, and 51% were positive in the winter when cows were housed. Positive samples contained on average 1 x 10^4 *Campylobacters* per gram of feces.

The high frequency of *Campylobacter* isolation in feces of cows suggests that contamination of bulk tank milk occurs primarily via feces of *Campylobacter*-carrier cows during harvesting and/or storage of raw milk (Waterman et al., 1984). To investigate environmental and animal sources in the dairy farm environment that could act as a reservoir or transient carriers of foodborne pathogens, a study was conducted in selected dairy farms located in East Tennessee. Although *C. jejuni* was not detected in samples of bulk tank milk, the organism was isolated frequently in lagoon water, fecal slurry/pats and bedding (Murinda et al., 2004b). These results support the concept that cattle feces are an important source of *Campylobacter* and that hygiene during milking is an important critical point to control contamination of raw milk with *Campylobacter.*

Equally important is the contamination of ground water with dairy farm organic effluents (*i.e.,* lagoon water). Hydrological evidence suggested a dairy farm was the source of water contamination with *C. jejuni*. Results from systematic microbiological monitoring of the polluted spring showed presence of *C. jejuni* and some strains isolated from water had identical biotypes with strains isolated from the dairy herd (Stanley et al., 1998). In another report, the occurrence of *Campylobacter* in fecal samples from a dairy herd and in a lake used as the source of cattle drinking water was studied. More cows were found *Campylobacter*-positive in the summer and fall when the water source was the nearby lake than during the winter when animals were housed inside and the water source was municipal chlorinated tap water. *Campylobacter jejuni* was isolated from most of the lake water samples. Serotyping of isolates with heat stable antigens and molecular typing by pulsed-field gel electrophoresis (PFGE) identified an animal that was chronically infected with *C. jejuni* sero-/PFGE-type PEN 0:6, 25/I/ND that most likely contaminated the lake water in the summer (Hanninen et al., 1998).

Listeria monocytogenes has also been isolated from cows with mastitis, but like *C. jejuni, L. monocytogenes* has not been isolated frequently and does not appear to be a significant cause of mastitis in dairy cows. In support of this contention, Jensen et al. (1996) examined quarter milk samples during a 23-year period from 1972 through 1994 for the presence of *L. monocytogenes*. During this time, 1,132,958 cows from 36,199 herds were sampled. The percentage of cows infected with *L. monocytogenes* varied from 0.01 to 0.1% (mean 0.04%). The percentage of herds with a *L. monocytogenes* infected cow ranged from 0.2 to 4.2% (mean 1.2%) showing a low but constant level of infection. A comparison of 33 *L. monocytogenes* isolates from bovine mastitis and 27 human clinical *L. monocytogenes* isolates showed that all bovine and 17 (63%) of the human isolates belonged to serogroup 1, whereas 10 (37%) of the human isolates belonged to serogroup 4. Ribotyping divided the 60 isolates into 16 different types, 7 of which were found among both the bovine and human types. The combination of typing methods showed that 26 (79%) bovine and 13 (48%) human isolates shared common types. This study showed that a low but constant percentage of Danish dairy herds have cows infected with *L. monocytogenes* and that some of the bovine types could be found among types causing human infections.

Listeria monocytogenes was isolated from the milk of two cows and two sheep with mastitis (Winter et al., 2004). Animals were observed over a 2 to 12 month period. Clinical examination of the udder, bacteriological examinations and determination of SCC of milk samples were performed monthly. All four cases resulted in subclinical mastitis characterized by an elevated SCC (0.8 - 10.1 x 10^6 cells/ml), persistent shedding of *L. monocytogenes* and a normal appearance of milk. Animals did not show systemic reactions, but all animals developed atrophy of the infected mammary gland. Histological examination revealed chronic interstitial mastitis with diffuse infiltration of lymphocytes, plasma cells and macrophages. All internal organs showed no abnormalities and no *L. monocytogenes* was isolated. *Listeria monocytogenes* was, however, isolated from the affected mammary parenchyma and from the mammary lymph node. Results of the bacteriological examination were confirmed by PCR and all *L. monocytogenes* isolates from the same animal were identical based on PFGE profiling.

Similar results were reported by Tzora et al. (1998) following experimental infection of ovine mammary glands with *L. monocytogenes*. No distinct variation in the pathogenicity of *L. monocytogenes* isolates was evident, all resulted in subclinical mastitis independent of their origin or serotype. A *L. innocua* isolate caused only a transient increase in milk SCC. After challenge, *L. monocytogenes* was isolated for 88 days from the milk of inoculated glands, and the milk SCC was > 1.0 x 10^6/ml. The organism was also isolated from mammary lymph nodes, but not from any internal organ of any inoculated ewe.

Bourry et al. (1995) compared results of experimental mastitis in dairy cows following intramammary inoculation of *L. monocytogenes* with two naturally occurring cases of bovine mastitis. Four strains of *L. monocytogenes*, two of serotype 1/2a and two of serotype 4b, were used for experimental infections and two diametrically opposed quarters of four cows were inoculated with 300 cfu. Bacteriological examination and SCC of quarter foremilk samples were performed weekly for at least 6 months after challenge. All inoculated mammary quarters developed chronic subclinical mastitis with occasional clinical episodes. Results were similar to those observed in natural *Listeria* mastitis. Four experimentally infected quarters were treated during lactation (gentamicin and cloxacillin), at drying off (cloxacillin), or at both times. Only one of four mammary quarters was cured after treatment only at drying off. All experimental and naturally infected animals were slaughtered and bacteriological examination was performed on liver, spleen, and supramammary, iliac and mesenteric lymph nodes. *Listeria monocytogenes* was isolated from supramammary lymph nodes of two experimentally and two naturally infected cows and from an iliac lymph node from one of the naturally infected cows. Epidemiological data were supported by serotyping, lysotyping and DNA macro-restriction analysis. Experimental *L. monocytogenes* mastitis was similar to naturally occurring cases of *L. monocytogenes* mastitis.

Reports on the prevalence of STEC associated with mastitis are rare. Stephan and Kuhn (1999) and Barrow and Hill (1989) indicated prevalence rates of STEC in *E. coli* mastitis of 2.75% (4 of

145) and 0.5% (1 of 237), respectively. Conversely, Cullor (1997) indicated the absence of Stx-producing *E. coli* from 500 cases of coliform mastitis that included *E. coli*. A recent study by Murinda et al. (2004a) demonstrated that of 105 *E. coli* mastitis isolates evaluated, all were *stx*-negative suggesting alternative virulence characteristics are involved in mastitis; only 4 of these isolates were positive for *eaeA* gene sequences. Thus, it would appear that STEC are rarely associated as a causative agent of bovine mastitis.

Reports on the prevalence of *Salmonella* associated with mastitis are also very rare. Spier et al. (1991) experimentally infected 5 post-parturient Holstein cows by intramammary inoculation of 5000 cfu of virulent *Salmonella dublin*. Rectal temperature, pulse and respiratory rates, milk yield, and milk quality as assessed by the California Mastitis Test (CMT) and SCC were recorded every 12 hours at milking. Bacteriologic cultures of foremilk quarter samples and feces were obtained daily, as were complete blood counts. ELISA titers for IgG and IgM recognizing *S. dublin* lipopolysaccharide (LPS) were obtained weekly on serum and quarter milk samples. All cows excreted *S. dublin* intermittently from infected quarters, but no changes were detected in rectal temperature, appearance of the mammary gland or secretions, CBC, milk yield, and pulse and respiratory rates. Somatic cell counts were modestly increased in infected quarters as compared with uninfected quarters; however, CMT scores after infection remained low, and were not significantly different from pre-infection scores. After infection, administration of dexamethasone resulted in signs of clinical mastitis and increased excretion of *S. dublin* from mammary quarters. One cow had necrotizing mastitis and *S. dublin* septicemia and was euthanized. In the four surviving cows, clinical improvement was observed after systemic gentamicin therapy and intramammary infusion with polymyxin B, but all cows continued to excrete *S. dublin* intermittently from one or more mammary quarters and occasionally from feces for the remaining period of observation. All infected cows demonstrated a rise in IgG and IgM ELISA titers recognizing *S. dublin* LPS in serum and milk. At necropsy (13 - 25 weeks postinfection), *S. dublin* was recovered only from mammary tissue or supramammary lymph nodes in three of four cows. In one cow, mammary gland and lymph node samples were negative for *S. dublin* despite positive milk cultures. In all cows, histopathologic examination revealed multifocal areas of chronic active mastitis and were similar to histopathologic findings from mammary gland carriers with naturally acquired *S. dublin* infection.

Food Safety and Public Health Implications

Pasteurization is regarded as an effective method to eliminate foodborne pathogens and other bacteria from milk. However, the increasing number of reports on detection of foodborne pathogens in pasteurized fluid milk and ready-to-eat dairy products clearly indicates that pasteurization alone is not the final solution for the control of milkborne pathogens. In addition, consumption of raw milk has been recognized as a major cause of foodborne diseases. Although the true incidence of milkborne disease in the United States is unknown, there are reports in which consumption of contaminated raw milk, faulty pasteurized milk, or consumption of dairy products adulterated with contaminated raw milk was linked directly to cases of human foodborne disease (Evans et al., 1996; Fashley et al., 1995; Fleming et al., 1985). For example, a high proportion of human infections caused by *C. jejuni* occurred through ingestion of untreated water, non-pasteurized milk, and faulty pasteurized milk contaminated with this foodborne pathogen (Evans et al., 1996; Fashey et al., 1995). Raw milk is also used to produce hard cheeses that are aged for more than 60 days such as Cheddar, Colby, Parmigiano and Provolone. Eleven foodborne disease outbreaks associated with cheese were reported in the U. S. from 1958-1991 (CDC, 1996; Johnson et al., 1990). Causative factors in cheese-related disease outbreaks were post-pasteurization contamination, faulty pasteurization equipment or procedures, and use of raw unpasteurized milk (Johnson et al., 1990). In 1999, FDA temporarily closed a cheese-making operation and recalled cheese that was supposedly contaminated with *L. monocytogenes*. The recall involved 135 pounds of Colby cheese made from raw milk. *Listeria monocytogenes* contamination showed up in tests conducted by federal health officials.

Outbreaks of human salmonellosis have also been linked to ingestion of raw milk contaminated with *Salmonella*. The 2002 - 2003 multistate (Illinois, Indiana, Ohio, and Tennessee) outbreak of *Salmonella* Serotype Typhimurium infections was linked to an Ohio dairy that comprised a working dairy farm, restaurant, snack bar, and petting zoo with goats, cows, calves, lambs, and pigs. The dairy was the only place in Ohio that sold raw milk in jugs and served raw milk and milk shakes made with raw milk legally to customers. A total of 62 persons had illness consistent with the case definition, including 40 customers, six household contacts, and 16 of 211 dairy workers. Patients were from four states (Illinois, Indiana, Ohio, and Tennessee). Of the 62 patients, 54 (87.1%) reported signs and symptoms of illness, which included diarrhea, cramps, fever, chills, body aches, bloody diarrhea, nausea, vomiting, and headache. A case-study including 40 case-patients from this outbreak and 56 controls revealed that only consumption of raw milk was significantly associated with illness. Consumption of other food items, visiting the petting zoo, and petting animals were not associated with illness. Five of 32 food samples tested including three raw skim milk samples, one sample of butter made from raw milk purchased by a customer, and one sample of cream were positive for *S.* Typhimurium that had a PFGE pattern that matched the strain outbreak pattern (CDC, 2003). These few examples suggest that although highly effective, milk pasteurization should not be considered as the absolute barrier to eliminate foodborne pathogens from milk, and that consumption of raw milk contaminated with one or more foodborne pathogens is a significant risk factor in the acquisition of human foodborne disease.

Although numerous studies have documented that foodborne pathogens of public health significance have been isolated from bulk tank milk and are capable of causing disease in humans, people continue to consume raw milk. Many farm families consume raw milk simply because it is a traditional practice and it is less expensive to take milk from the bulk tank than buying pasteurized retail milk (Hegarty et al., 2002). Some believe that raw milk has a higher nutritional value than pasteurized milk (Hegarty et al., 2002). A study by Headrick et al. (1997) showed that people with less than a high school education were more likely to consume raw milk than those who had completed high school, suggesting that level of education may influence a person's choice to consume raw milk.

Rohrbach et al. (1992) reported that 68 of 195 (34.9%) dairy producers in East Tennessee and Southwest Virginia consumed raw bulk tank milk produced on their farm. Of the bulk tanks from which raw milk was consumed by dairy producers, 25% (17 of 68) contained one or more species of *L. monocytogenes*, *C. jejuni*, *Y. enterocolitica* and *Salmonella* (Rohrbach et al., 1992). On farms where producers did not consume raw milk, 32% (40 of 127) of bulk tanks were contaminated with one or more of the above zoonotic pathogens, and bulk tank milk quality was not significantly different from those dairy producers who reported that they consumed raw milk from the bulk tank (25%, 17 of 68). The prevalence of consumption of bulk tank milk containing one or more potential human pathogenic bacteria among all dairy producers was 17/195 or 8.7%. Jayarao and Henning

(2001) reported that 79 of 131 (60%) dairy producers in eastern South Dakota and western Minnesota consumed raw bulk tank milk produced on their farm. Of the 79 dairy producers who consumed raw milk, 21 (26.6) contained one or more species of *L. monocytogenes*, *C. jejuni*, *Y. enterocolitica*, STEC, and *Salmonella*. There was no significant difference in the incidence of pathogenic bacteria in raw milk of dairy producers who did and did not consume raw milk. Thus, based on the limited information available, it would appear that numerous persons in the rural community consume raw unpasteurized bulk tank milk.

Using data on raw milk consumption by dairy producers of 60% from the survey conducted by Jayarao and Henning (2001), and assuming that 25% of bulk tanks contain one or more potential human pathogens based on data reported by Rohrbach et al. (1992) and Jayarao and Henning (2001), the prevalence of consumption of contaminated bulk tank milk would be 15%. Nationally, an estimated 14,000 dairy farm families could be exposed daily to several potential human pathogens via consumption of raw unpasteurized bulk tank milk. This estimate DOES NOT include consumption of raw milk by farm employees and their immediate families, neighbors, and other members of the rural community that have access to raw milk produced on these farms. Thus, risk exposure of people in the rural community to potential pathogenic bacteria capable of causing disease in humans via consumption of raw unpasteurized milk can be very high. These data also emphasize the need for educational efforts on health risks associated with consumption of raw unpasteurized milk.

In addition to direct consumption of contaminated raw milk, introduction of raw milk contaminated with foodborne pathogens into dairy processing plants represents an important risk of contamination of milk products that could lead to exposure of consumers to pathogenic bacteria. Although milk pasteurization is regarded as an effective method to eliminate foodborne pathogens, some dairy products do not undergo pasteurization (i.e, specialty cheeses). Furthermore, pathogens such as *L. monocytogenes* survive and thrive in post-pasteurization processing environments thus leading to recontamination of dairy products. These two significant exposure pathways pose a risk to the consumer from direct exposure to foodborne pathogens in unpasteurized dairy products as well as dairy products which are re-contaminated in the post-pasteurization processing environment. The increasing number of incidences in which foodborne pathogens are detected in fluid milk and ready-to-eat dairy products clearly indicate that pasteurization is not the ultimate tool to control milkborne pathogens. It is likely that fecal and foodborne pathogen contamination occurs during the harvesting of raw milk (i.e., milking, collection, and storage) and the farm environment likely plays a major role in the presence of foodborne pathogens in bulk tank milk. Reducing the potential for contamination during harvesting of milk should result in the reduction of foodborne pathogens in raw milk.

Introduction of *L. monocytogenes* into food processing plants results in reservoirs that are difficult to eradicate. For instance, biofilms are a constant issue in food processing environments. *Listeria monocytogenes* survived for extended periods on stainless steel and buna-n rubber, materials commonly used in food-processing equipment. Some components in the rubber inhibited growth of the organism on buna-n, but also affected the efficacy of sanitizers on biofilm inactivation. These conditions lead to the persistence of low numbers of *L. monocytogenes* on equipment surfaces making eradication difficult (Wong, 1998). The level of contamination of milk processing plants was investigated by several research groups. In a survey conducted in frozen milk product plants involving 39 plants in California, *L. monocytogenes* was the only species recovered from 5 (12.8%) plants. Observations of this study indicated that the type of product received and type of pasteurization did not influence recovery of *Listeria* from a plant. However, a significant association with size of the plant and recovery of *Listeria* was observed. Also, it was observed that the level of sanitation and extent of environmental contamination control program (ECCP) were not associated with recovery of *Listeria* from a plant. Still, the rate of recovery of *Listeria* from plants with above average sanitation and excellent or moderate ECCP was 6.81%, whereas the recovery rate from plants with below average sanitation and slight or no ECCP was 27.5%. Interestingly, the highest recovery rates of *Listeria* in frozen milk product plants were in batch flavoring, freezing and ingredient blending, and packaging filling areas (Walker et al., 1991). In a study conducted in 21 dairy processing plants in Vermont, 80 of 378 sites (21.2%) were identified as *Listeria*-positive and of these, 35 (43.8%) were positive for *L. monocytogenes* (Pritchard et al., 1995).

Mycobacterium avium subsp. *paratuberculosis* (MPTB), the etiologic agent of Johne's disease in dairy and beef cattle and sheep, is considered by some to be an emerging foodborne pathogen. This concern is based on reports that have shown that Crohn's disease in human patients bears a clinical resemblance to Johne's disease. Current evidence neither supports or refutes a mycobacterial cause of Crohn's disease, but evidence does suggest that either a subset of human patients are infected with MPTB or that the ulcerated tissues of Crohn's disease patients selectively harbor MPTB acquired as an environmental contaminant or opportunistic pathogen (Bannantine et al., 2004; Harris and Barletta, 2001; Sechi et al., 2001). Milk from dairy cows with Johne's disease could be a potential vector in the transmission of MPTB from cattle to humans based on evidence demonstrating that MPTB can be shed and isolated from raw milk and that MPTB may survive pasteurization (Grant et al. 2002a; 2002b; Millar et al., 1996).

While the role of MPTB is firmly established in Johne's disease in cattle and sheep, its role in Crohn's disease is less clear (Bannantine et al., 2004). The need for a definitive answer to this important question has been recognized by the scientific community and is currently being investigated by several research groups. The biology of MPTB makes its manipulation in the laboratory and development of rapid and accurate diagnostic methods difficult. New detection methods are needed to definitively answer questions surrounding MPTB as a foodborne pathogen and if this pathogen is a contributing factor in Crohn's disease. Recent advances in the study of the MPTB genome have lead to the identification of specific DNA sequences and protein antigens that may allow reliable detection of MPTB (Bannantine et al., 2004). Improving the detection of MPTB would help to answer fundamental questions on the epidemiology and pathogenesis of MPTB including its role in foodborne illness. This topic and its implications will be reviewed elsewhere (Stabel, 2005) at the Conference Symposium on Does High Somatic Cell Counts in Milk Constitute a Human Health Risk?

Conclusions

The dairy industry should be concerned about food safety because: (1) bulk tank milk contains several foodborne pathogens that cause human disease, (2) outbreaks of disease in humans have been traced to the consumption of raw unpasteurized milk and have also been traced back to pasteurized milk, (3) raw unpasteurized milk is consumed directly by dairy producers and their families, farm employees and their families, neighbors, etc., (4) raw unpasteurized milk is consumed directly by a much larger segment of the population via consumption of several types of cheeses including ethnic cheeses manufactured from unpasteurized raw milk, (5) entry of foodborne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms and subsequent contamination of processed food products, (6) pasteurization may not destroy ALL foodborne pathogens in milk, and (7) faulty pasteurization will not destroy all foodborne pathogens.

Information presented in this review support the model in which the presence of the pathogen depends on ingestion of contaminated feed followed by amplification in bovine hosts and fecal dissemination in the farm environment. The final outcome of this cycle is a self maintained reservoir of a pathogen that can reach the human population by direct contact, ingestion of raw contaminated food (raw milk or cheese made with raw milk), or contamination during the processing of food. Isolation of strains with similar biotypes from dairy farms and human cases and outbreaks substantiate this hypothesis.

The challenges to providing a safe and nutritious food supply are complex because all aspects of food production – from farm to fork – need to be considered. Given the considerable national/international demand for food safety and the formidable challenges of producing and maintaining a safe food supply, food safety research and educational programs has taken on a new urgency. As the system of food production and distribution changes, the food safety system needs to change with it. A strong science-based approach that addresses all the complex issues involved in continuing to improve food safety and public health is necessary to prevent foodborne illnesses. Not only must research be conducted to solve complex food safety problems, results of that research must be communicated effectively to producers and consumers. Research and educational efforts identifying potential on-farm risk factors will better enable dairy producers to reduce/prevent foodborne pathogen contamination of dairy products leaving the farm. Identification of on-farm reservoirs could aid with implementation of farm-specific pathogen reduction programs. Foodborne pathogens, mastitis, milk quality and dairy food safety are indeed all interrelated. A safe, abundant and nutritious milk and meat supply should be the goal of every dairy producer in the world.

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